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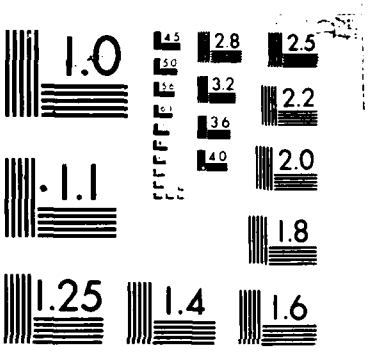

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The purpose of the requested instrumentation was to support and facilitate new and expanded research efforts in basic membrane studies, predictive toxicology and tropical medicine. The specific facility requested included a preparative free-flow electrophoresis unit with a u.v. scanner and a medium resolution electron microscope to monitor and evaluate the separations achieved. A major anticipated use was in support of a project "Early Phase Interactions of Toluene with Membranes: Structural and Functional Evaluation" funded by the Air Force Office of Scientific Research.

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SCHOOL OF PHARMACY AND
PHARMACAL SCIENCES

28 November 1986

Lt. Col. Lorris Cockerham
Program Manager, Life Sciences Directorate
Air Force Office of Scientific Research/NL
Department of the Air Force
Bldg. 410, Bolling Air Force Base
Washington, DC 20332

Dear Lt. Col. Cockerham

Enclosed is the Final Technical Report for our AFOSR-84-0297 project "Request for Instrumentation." My apologies for the initial confusion and the slight additional delay in forwarding the report to you.

The subject support was much appreciated and as you will note from the considerable progress cited has developed into an outstandling useful and productive facility. Not only has our AFOSR project been aided by the facility but other projects as well and we anticipate continued applications to defense-related research.

Best regards

D. James Morre
Dow Distinguished Professor
of Medicinal Chemistry

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cc: Cheryl A. Maurana, DSP

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9 DEC 1986

Final Technical Report

AFOSR-84-0297

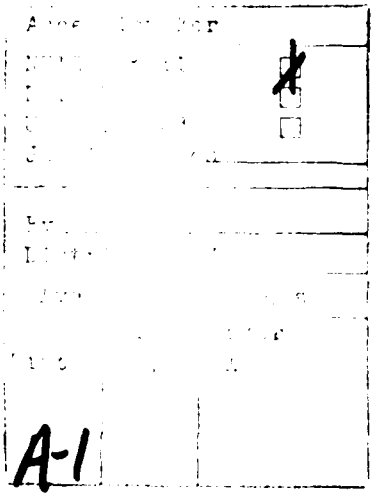
REQUEST FOR INSTRUMENTATION

To: Lt. Col. Lorris Cockerham
Program Manager, Life Sciences Directorate
Air Force Office of Scientific Research/NL
Department of the Air Force
Bldg. 410, Bolling Air Force Base
Washington, D.C. 20332

From: Dr. Dr. James Morré, Ph.D.
Dow Distinguished Professor of Medicinal Chemistry
Department of Medicinal Chemistry
School of Pharmacy
Purdue University
West Lafayette, IN 47907

Principal Investigator

17 November 1986
Date



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Purpose: The purpose of the requested instrumentation was to support and facilitate new and expanded research efforts in basic membrane studies, predictive toxicology and tropical medicine. The specific facility requested included a preparative free-flow electrophoresis unit with a u.v. scanner and a medium resolution electron microscrope to monitor and evaluate the separations achieved. A major anticipated use was in support of a project "Early Phase Interactions of Toluene with Membranes: Structural and Functional Evaluation" funded by the Air Force Office of Scientific Research.

Equipment Purchased:

VAP-22 Preparative Free-Flow Electrophoresis (Bender and Hobein, Munich, FRG).

Zeiss EM/109 Transmission Electron Microscope (Carl Zeiss, Inc., Oakbrook, IL).

Progress: The equipment was used to establish a cell/organelle subfractionation facility consisting of preparative free-flow electrophoresis interfaced with transmission electron microscopy to monitor and evaluate the separations achieved. The facility and equipment presently is located in the Life Sciences Research Building of the Main Campus of Purdue University.

The major use thus far has been in support of a project "Early Phase Interactions of Toluene with Membranes" funded through the United States Air Force Office of Scientific Research. Under this project, ultrastructural, biochemical and molecular approaches have been applied to problems of how toluene influences membrane structure and function. The basic hypothesis under test is that toluene may intercalate into membranes and disrupt membrane organization through a disturbance particularly of boundary lipids known to be critical to membrane function and stability. In this connection, free-flow electrophoresis provides a rapid and convenient means to isolate membranes especially from cultured cells. All phases of the study have relied heavily on electron microscopy. Major advances have come as well in the purification and concentration of membrane located receptors potentially important to rapid developments in the area of molecular electronics and sensor development for environmental toxicants including antipersonnel agents important to military defense.

With the technique of free flow electrophoresis, mixtures of membranous cell components to be separated are introduced as a fine jet into a separation buffer moving across the field lines of an electric field. It is a powerful separation tool developed over the past decade largely in West Germany and currently of very limited availability to investigators in the United States. The separation technique is one of the few procedures capable of separating parasites from host cell components, of separating infected or diseased from normal cells of the same cell type, of separating cell types based on pathological differences not related to infectious agents, and of complete subfractionation of cell surface and internal membrane compartments in a single preparative step. The procedure is amenable to continuous batch operation with preparation of gram quantities of materials but highly dependent upon morphological criteria for routine monitoring of separation efficacy. Hence, the requirement that the preparative free-flow electrophoresis instrument be combined with a transmission electron microscope.

Electron microscopy is the method of choice for routine monitoring of membrane preparations since only small amounts of material are required, one obtains simultaneous estimates of fraction composition, purity and integrity, and a single analytical method can be applied simultaneously to a wide range of separation problems. Continuous operations of the facilty during the past year has generated approximately 200 electron microscope samples per week. This number of samples has required between 20 and 30 or more hours of microscope time per week for evaluation plus the services of a full-time technician for processing and thin sectioning of specimens.

Direct cost sharing was provided through the provision of ancillary equipment for specimen preparation for electron microscopy, a second free-flow electrophoresis unit and needed centrifuges and installation costs with an estimated total of about $65,000.

Indirect cost sharing was provided through approximately 30% of effort by the principal investigator (including time from existing research projects requiring use of the facility) to its operation and development plus technical support. A full-time electrophoresis technician operates and maintains the preparative free-flow electrophoresis system and an electron microscope technician is responsible for specimen preparation and photographic processing. Those who have utilized the facility include 8 graduate students, 5 postdoctoral associates and 10 visiting scientists from other universities. Each of the graduate students and postdoctorals as well as many of the visiting scientists have received advanced training in cell and membrane fractionation as a result of the facility.

Purdue University staff who have used the facility extensively or have benefited from its operation through students and associates other than the principal investigator include:

Dr. Frederick L. Crane, Department of Biological Sciences

Dr. Linda B. Jacobsen, Director, Mammalian Cell Culture Laboratory

Dr. Charles Bracker, Department of Botany and Plant Pathology

Dr. Robert Geahlen, Department of Medicinal Chemistry and

additionally

Dr. Hilton H. Mollenhauer, Veterinary Toxicology Laboratory, College Station, TX, a co-investigator on two of the projects supported by the facility, also has been a major user during the past six months as a Visiting Scientist on the Purdue University campus.

A major accomplishment derived from the availability of the facility has been the demonstration of the general applicability of free-flow electrophoresis as a new technology based on surface charge for separation of biological membranes.

The apparatus provided were among the first to be generally available in the United States and many scientists already have visited our facility and have left with plans to develop similar facilities of their own. The findings were summarized as part of a very successful conference on new separation

methods held in Heidelberg, FRG, October 1-4, 1986 and sponsored by the United States Air Force Office of Scientific Research during which demonstrations, discussion and applications of the new methodology formed the primary basis for the meeting.

Publications: (partial listing of works published since the facility has been operational):

1. Sandelius, A., C. Penel, G. Auderset, A. Brightman, K. Safranski, H. Greppin and D. J. Morre. 1986. Isolation of plasma membrane and tonoplast from the same homogenates of plant cells by free-flow electrophoresis. In: A. J. Trewavas, ed. Molecular and Cellular Aspects of Calcium in Plant Development. Plenum Press, New York. pp. 353-354.

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3. Sandelius, A. S., C. Penel, G. Auderset, A. Brightman, M. Millard and D. J. Morre. 1986. Isolation of highly purified fractions of plasma membrane and tonoplast from the same homogenate of soybean hypocotyls by free-flow electrophoresis. Plant Physiol. 81: 177-185.

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5. Morre, D. J., M. Paulik and D. Nowack. 1986. Transition vesicle formation in vitro. Protoplasma 132: 110-113.

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10. Morre, D. J., P. Navas and F. L. Crane. In press. Isolation, purification, evaluation and quantitation of plasma membranes from tissues and cultured cells: Methods and applications to cell-free systems for analyses of growth processes. In: J. Ramirez, ed. Proc. U.S.-Spain Joint Symposium on Electron Transfer Constituents of the Eukaryotic Plasma Membrane, Madrid, 1986.

11. Crane, F. L., H. Löw, I. L. Sun, P. Navas and D. J. Morre. In press. Redox control of cell growth. In: J. Ramirez, ed. Proc. U.S.-Spain Joint Symposium on Electron Transfer Constituents of the Eukaryotic Plasma Membrane, Madrid, 1986.

12. Navas, P., I. L. Sun, F. L. Crane and D. J. Morre. In press. Changes in the pyridine nucleotide pools of HeLa cells in response to growth promoting agents. In: J. Ramirez, ed. Proc. U.S.-Spain Joint Symposium on Electron Transfer Constituents of the Eukaryotic Plasma Membrane, Madrid, 1986.

13. Minnifield, N., K. E. Creek, P. Navas and D. J. Morre. In press. Distribution of β-hexosaminidase and oligosaccharide processing enzymes across the polarity axis of rat liver Golgi apparatus based on free-flow electrophoresis. Eur. J. Cell Biol.

14. Morre, D. J., A. Brightman, G. Scherer, B. vom Dorp, C. Penel, G. Auderset, A. S. Sandelius and H. Greppin. In press. Highly purified tonoplast fractions by preparative free-flow electrophoresis. In: Proc. 1st International Workshop on Plant Vacuoles, Sophia-Antipolis, France, July 6-11, 1986.

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16. Penel, C., G. Auderset, S. Kiefer, A. Sandelius, A. Brightman, H. Greppin and D. J. Morre. In Press. Plasma membrane and tonoplast fractions isolated from spinach leaves by preparative free-flow electrophoresis. In: P. K. Stumpf. Metabolism Function and Structure of Plant Lipids (Proc. 7th Intern. Symp. Met., Funct. and Struct. Plant Lipids, Davis, CA, July 27-August 1, 1986) Plenum, New York.

17. Morre, D. J., F. L. Crane, I. L. Sun and P. Navas. In Press. The role of ascorbate in biomembrane energetics. Ann. N.Y. Acad. Sci.

18. Matyas, G. R. and D. J. Morre. Submitted. Subcellular distribution and biosynthesis of rat liver gangliosides. J. Biol. Chem.

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20. Brightman, A., P. Navas, N. Minnifield and D. J. Morre. In preparation. A function for thiamine pyrophosphatase of Golgi apparatus. Biochim. Biophys. Acta.

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22. Sun, I. L., P. Navas, F. L. Crane, D. J. Morre and H. Löw. In preparation. NADH diferric reductase activity in liver plasma membranes. FEBS Lttrs.

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24. Barr, R., A. S. Sandelius, F. L. Crane and D. J. Morre. In preparation. Redox reactions of tonoplast and plasma membranes isolated from soybean hypocotyls by free-flow electrophoresis. Plant Physiol.

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26. Mollenhauer, H. H., D. J. Morre and D. Pikaard. In preparation. An ultrastructure evaluation of toluene toxicity using cultured mammalian cells. Cell Tissue Res.

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3. Navas, P., A. Brightman, N. Minnifield and D. J. Morre. 1985. A function for the thiamine pyrophosphatase of Golgi apparatus. J. Cell Biol. 101:58a.

4. Crane, F. L., I. L. Sun, D. J. Morre and H. Löw. 1985. Growth control by transmembrane and differic transferrin reductase. J. Cell Biol. 101:483a.

5. Sandelius, A. S., C. Penel, G. Auderset, K. Safranski, H. Greppin and D. J. Morre. 1985. Isolation of plasma membrane and tonoplast membrane fractions of soybean hypocotyls using preparative free-flow electrophoresis. Physiol. Plant. 64:27a.

6. Sandelius, A. S. and D. J. Morre. 1985. Auxin stimulates turnover of a membrane lipid, phosphatidylinositol, in soybean membranes. Physiol. Plant. 64:21a.

7. Barr, R., A. S. Sandelius, D. J. Morre and F. L. Crane. 1985. Redox activities of highly purified tonoplast and plasma membranes by free-flow electrophoresis and by phase partition. Plant Physiol. 77 (Suppl.):145.

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10. Burón, I. M., P. Navas, G. García-Herduyo and D. J. Morre. 1986. Plasma membranes from amphibian epidermis: Isolation and subfractionation by preparative free-flow electrophoresis. J. Cell Biol.

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13. Morre, D. J., A. S. Sandelius, R. Barr and F. L. Crane. 1986. Similarities and differences comparing tonoplast and plasma membrane compartments based on plant cell fractionation studies. Plant Physiol. 80:101

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19. Morre, D. J. 1986. Preparative free-flow electrophoresis: Applications to exocytotic compartments. Abstracts. Special Meeting, European Molecular Biology Laboratory, Cell-free Analysis of Membrane Traffic, Heidelberg, FRG, October 1-4, 1986. p. 2.

20. Minnifield, N., K. Safranski and D. J. Morre. 1986. Phosphomannosyl receptor binding is localized in vesicles separated by preparative free-flow electrophoresis from rough endoplasmic reticulum preparations of rat liver. Abstracts. Special Meeting, European Molecular Biology Laboratory, Cell-free Analysis of Membrane Traffic, Heidelberg, FRG, October 1-4, 1986. p. 18.

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22. Auderset, G., A. Sandelius, C. Penel, A. Brightman, H. Greppin and D. J. Morre. 1986. Plasma membrane and tonoplast fractions from spinach leaves by preparative free-flow electrophoresis. Abstracts. Special Meeting, European Molecular Biology Laboratory, Cell-free Analysis of Membrane Traffic, Heidelberg, FRG, October 1-4, 1986. p. 10.

23. Navas, P., M. Paulik and D. J. Morre. Preparative free-flow electrophoresis for isolation of plasma membranes from cultured cells. Abstracts. Special Meeting, European Molecular Biology Laboratory, Cell-free Analysis of Membrane Traffic, Heidelberg, FRG, October 1-4, 1986. p. 18.

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27. Canut, H., G. Auderset, A. Brightman, C. Penel and D. J. Morre. Submitted. Plasma membrane and tonoplast vesicles resolved by free-flow electrophoresis into subpopulations of different sidedness. Abstracts 1987 UCLA Symposium on Molecular and Cellular Biology, Plant Membranes: Structure, Functions, Biogenesis, Park City, Utah. February 8-13, 1987.

28. Brightman, A., R. Barr and D. J. Morre. Submitted. Purification of an auxin-stimulated NADH oxidase from plasma membrane of soybean. Abstracts 1987 UCLA Symposium on Molecular and Cellular Biology, Plant Membranes: Structure, Function, Biogenesis, Park City, Utah. February 8-13, 1987.

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